

ABSTRACT OF THE DISCLOSURE

The present invention relates to a method of detecting single nucleotide polymorphisms by providing a target nucleic acid molecule, an oligonucleotide primer complementary to a portion of the target nucleic acid molecule, a nucleic acid polymerizing enzyme, and a plurality of types of nucleotide analogs. The target nucleic acid molecule, the oligonucleotide primer, the nucleic acid polymerizing enzyme, and the nucleotide analogs, each type being present in a first amount, are blended to form an extension solution where the oligonucleotide primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site. The oligonucleotide primer in the extension solution is extended by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide primer at the active site. This forms an extended oligonucleotide primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site. The amounts of each type of the nucleotide analogs in the extension solution after the extending step are then determined where each type is present in a second amount. The first and second amounts of each type of the nucleotide analog are compared. The type of nucleotide analog where the first and second amounts differ as the nucleotide added to the oligonucleotide primer at the active site is then identified. The steps of extending, determining the amounts of each type of the nucleotide analog, comparing the first and second amounts of the nucleotide analog, and said identifying the type of nucleotide analog added may be repeated, either after repeating the blending with the extended oligonucleotide primer or after determining the amounts of each type of dideoxynucleotide or dideoxynucleotide analog remaining in the extension solution as the new first amount. As a result, the nucleotide at the active site of the target nucleic acid molecule is determined. Also disclosed is an apparatus and composition for carrying out this method.